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# PRELIMINARY TRIALS ON CULTIVATION OF *RHINOSPORIDIUM SEEBERI*

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# PRELIMINARY TRIALS ON CULTIVATION OF *RHINOSPORIDIUM SEEBERI*

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*Rhinosporidium seeberi*, the causative orgnism of reinosporidiosis in man, cattle, horses, mules and pigs has not been successfully cultivated so far. During the period of study of 4 years, Kanyakumari district of Tamil Nadu was found to be the high incidence area for both human and bovine rhinosporidiosis. Attempts were made to cultivate the fungus on routine media like Sabouraud's agar, and Tryptose agar both with and without carbondioxide but were not successful. So preliminary studies were carried out to sustain the growth and also to study the cyclic growth of sporangium during the period of growth.

## Materials and Methods

The raft technique (Merchant *et al.*, 1963) was employed. Sterile "Falcon" plastic petri dishes were taken and sterile stainless steel wire mesh 1 cm square was placed in the center. The human rhinosporidial nasal polyps was frozen at -20°C and pieces about the size of 5 mm width and 4 mm thickness were cut. The rhinosporidial polyps was washed well in Hanks basal salt solution and placed on the wire mesh. Sterile filter paper wicks were placed on either side of the wire mesh. Tissue culture medium 199 (Difco) with foetal calf serum (Difco) was added in sufficient quantities to wet the filter paper wick, but not so as to immerse the polyps.

## Results and Discussion

Throughout the period of observation (15 days) the pH of the medium did not change There was no contamination. The tissue was alive, throughout the period (Fig.). Spores were seen in tissues and in culture medium.

Rao (1938) reported about the successful cultivation of *Rhinosporidium seeberi* on Sabouraud's agar with dung juice and horse dung agar with gland juice smeared over it. But this growth was identified as *Spicaria sp.* Sathyanarayana (1960) failed to culture the organism either in developing eggs or in artificial media with amino acids, salts and vitamin or in high aqueous vapour tension and in high carbondioxide tension. Reddy and Lakshminarayan (1962)

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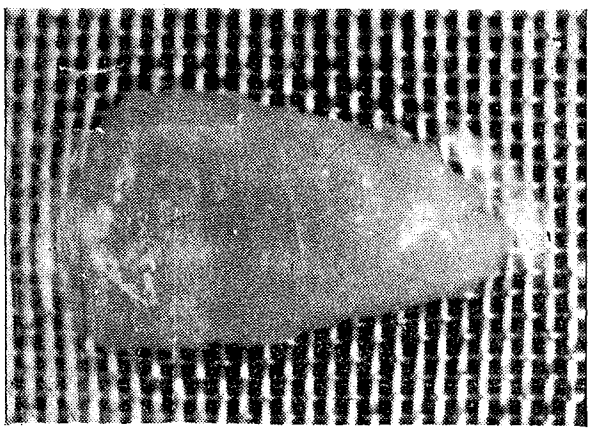

A piece of nasal polyp on a stainless steel grid.

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tried to cultivate the organism in Sabouraud's agar, blood agar, Lowenstin-Jensen medium and, Beef heart infusion agar, but failed to cultivate *Rhinosporidium seeberi*. Datta (1963) tried to cultivate the organism on carrot slants, and goat nasal tissue. But he isoloted *Aspergillus sp.* Gupta (1959) stated that *Rhinosporidium seeberi* could not be cultivated in Sabouraud agar or itr various modifications. Grover (1970) reported about the maturation of spores and sporangium in biopsy materials placed in synthetic liquid tissue culture mediun 199. Emmons *et al.* (1970), Jungerman and Schwartzman (1972), Rao *et al.* (1975), Kuttin and Baum (1980) have reported their inability to cultivate *Rhinosporidium seeberi*

In our attempts we tried to maintain the rhinosporidial polyps for a period of 15 days, to observe the production, multiplication and maturation of spores and sporangia. We were able to keep the polyps alive for 15 days. Further trials are being undertaken to cultivate the specimen in animal and plant tissue cultures and also adapt other culture techniques.

Van Breuseghem (1958) suggested that in India the disease affects mostly growing young adults, employed in lifting sand and gravel form riverbed and also the rinosporidium may infect fish which may accidentally attack man and animals. Kannan Kutty *et al.* (1963) suggested that as the conditions to cultivate the organism must very closely resemble the human blood nutrients, bio chemical nutrients, the Raft-technique was found ideal to mimic most of the conditions.

Emmons *et al* (*loc. cit.*) suggested that *Rhinosporium seeberi* grows either as a sapprobe or as a parasite of fish or water insects, but recognised only in tissues of animals and man.

Till now the possibility of intermediate stage has not been identified. Our study showed that the mature spores and sporangia remained as such without any visible differentiation. The intra vital staining of the polyps before and after organ culture showed that the polyps was viable throughout.

## Summary

1. Rhinosporidial polyps were maintained in Tissue culture medium 199 (Difco) with fetal calf serum for a period of 15 days by using Raft technique.
2. The pH of the medium did not change during the period.
3. Spores were seen in the tissues and in culture medium.
4. Rhinosporidial polyps were alive throughout.

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